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IS THE LEVEL OF IGG ANTIBODIES AGAINST PERTUSSIS TOXIN SUFFICIENT IN VACCINATED CHILD POPULATION?

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A b s t r a c t

The aim of the study is to evaluate the level of antibodies against pertussis toxin in a sample of vaccinated children in relation to the recent changes in the vaccination scheme in the Czech Republic. In 122 children born in 2000–2006, the level of IgG antibodies against pertussis toxin was examined using the ELISA method. These children received at least three doses of the vaccine, more than four weeks prior to blood collection. The type of the vaccine and the period of time after the last vaccination were observed. In the whole sample of vaccinated children, who received at least three doses of the vaccine, only 37 had a positive level of antibodies in the time period of 1–42 months after the last vaccination. The difference between the whole-cell vaccine and the acellular vaccine was not statistically significant. The period of time after the last vaccination was in inverse proportion to the number of children with a positive level of antibodies. With regard to the increasing incidence of pertussis in the Czech Republic it would be rewarding to initiate a discussion about the booster dose with the acellular vaccine against pertussis at the age of 9–14 years.

K e y w o r d s

Pertussis, Vaccination, Antibodies

A b b r e v i a t i o n s u s e d

PT, Pertussis toxin; FDA, Food and drug administration; ELISA, Enzyme-Linked Immunosorbent Assay; OR, Odds ratio ; OD. Optical density; BP, Bordetella pertussis; VE, Virotech units; WHO, World Health Organisation

INTRODUCTION

Pertussis is a highly contagious, vaccine-preventable infectious disease. It is defined, according to the WHO, as a cough lasting longer than three weeks with a parallel confirmation by the laboratory finding. Pertussis is caused by *Bordetella pertussis* (BP), a small coccoid rod, which is encapsulated and carries fimbriae. Bordetellas adhere to the mucous tissue of the upper respiratory tract and cause the disease by releasing their toxins. One of the most important toxins is pertussis toxin (PT). This toxin is highly specific to the infection of BP and there are no cross-reactivities described (*1, 2*).

The immune response is directed towards different antigens, but the reaction against PT is one of the most reliable. After the infection (vaccination), antibodies of all classes are developed. IgG antibodies occur in the serum 2–3 weeks after immunisation at the earliest and their level increases. The concentration of the specific IgG antibodies decreases again in the majority of the population below the level of 125 IU/ml (referring to FDA) within one year. An IgG-anti-PT concentration above the level of 125 IU/ml occurs in only approximately 1 % of the population (*3*).

Despite the high vaccination coverage in the Czech Republic, a growing incidence of pertussis has been reported in recent years. The history of vaccination against pertussis in the Czech Republic dates from 1958, when regular vaccination was introduced and whole-cell vaccines were used. Afterwards, the number of reported cases declined rapidly. The vaccination scheme consisted of three doses – the first dose was given from the ninth week, the second dose 6–8 weeks after the first dose, and the third dose was applied 6–8 months after the second dose. In 1994 the vaccination scheme was changed. The first 3 doses were applied from the ninth week in 1–2 months' intervals, the fourth dose was given in 18–20 months, and the fifth dose at five years of age (*4*). The acellular vaccines were introduced in 1999. However, the vaccination was free of charge only for infants with immunological or neurological indication. In 2004, the booster dose with the whole-cell vaccine at the age of 5 was replaced by the acellular one. Since 2007 acellular vaccines have been used for all doses free of charge.

The main difference between a whole-cell and an acellular vaccine is that the whole-cell vaccine contains more than 3000 antigens. Some of them are responsible for its higher reactogenicity. The most serious side effects are neurological ones (*5*). On the other hand, the acellular vaccine (in the Czech Republic the three-component type is used) contains pertussis toxin, filamentous haemagglutinin, and pertactin. That relates to its higher immunogenicity and the lower number of adverse effects (*6, 7*).

The aim of this study is to evaluate the level of IgG antibodies against pertussis toxin in a sample of vaccinated children.

MATERIALS AND METHODS

In 122 vaccinated children born 2000–2006 the level of IgG antibodies against PT was examined. The sera were processed and examined in a private immunoallergological laboratory in Brno under the supervision of qualified staff. The sera were collected for another indicated examination. The children received at least 3 doses of the vaccine more than 4 weeks prior to blood collection. The type of the vaccine used and the period of time after the last vaccination were observed. Seventy-two children within 12 months after the last vaccination were divided into two groups according to the type of the vaccine (whole-cell, acellular). Furthermore, 74 children who received the whole-cell vaccine were divided into three groups according to the period of time after the last vaccination (1–12 months, 13–35 months, 36–42 months after the last vaccination).

The level of IgG antibodies was evaluated using an enzyme-linked immunosorbent assay (ELISA). For this study, ELISA test-kits from the VIROTECH company were used. The evaluation of the level of IgG antibodies is semiquantitative by using cut-offs. The concentration is measured in VIROTECH units (VE).

$$\text{VE (sample)} = \frac{\text{Optical density (OD) (sample)}}{\text{OD (cut-off)}}$$

Fig. 1

Table 1
Interpretation of results in VE units
The data was statistically analysed using the Fisher exact test and the Pearson chi-square test

VE ‹ 9		Negative	
VE 9–11	36–44 IU/ml (FDA)	Borderline	Persistent antibodies of recent infection
			Antibodies of starting infection
			Vaccination antibodies
VE › 11		Positive	Indicator of acute or recent infection
			Antibodies due to vaccination *(impossible to differ postvaccination antibodies from postinfectious ones)*
VE › 18	›125 IU/ml (FDA)	Infection	Acute infection
			Vaccination no longer than 12 months ago

RESULTS

Only 18 children out of 48 vaccinated with the acellular vaccine 1–12 months prior to blood collection had positive levels of IgG antibodies against PT. In the group of children vaccinated with the whole-cell vaccine under the same conditions, 10 out of 24 had positive levels of antibodies. The difference between these two groups is not statistically significant (OR=1.19, p=0.8).

Table 2
Children 1–12 months after last vaccination

Type of vaccine	n=72	Positive	Negative
Acellular	48	18	30
Whole-cell	24	10	14

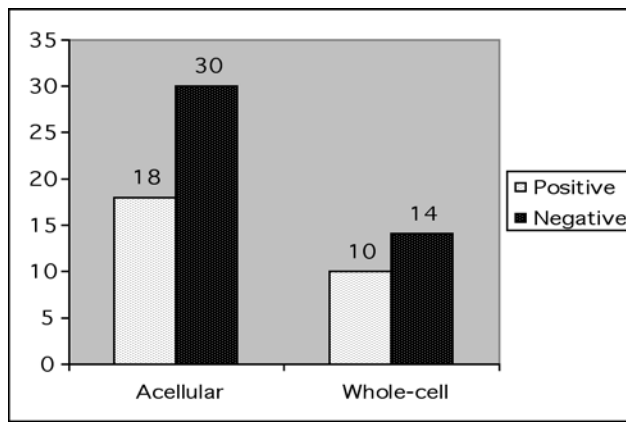

Fig. 2

Positive levels of IgG antibodies against PT in children 12–35 and 36–42 months after the last vaccination with whole-cell vaccines (31 and 19) were noticed only in 7 and 2 children, respectively. The difference between these two groups and the group 1–12 months after the vaccination with the whole-cell vaccine is statistically significant (OR=3.25, p=0.005), which means that the longer is the period of time

after the vaccination, the more often the antibodies are negative. In the group of children vaccinated with the whole-cell vaccine, we diagnosed 5 cases of pertussis. These children complied with the criteria of pertussis according to the WHO – they had a spasmodic cough lasting longer than three weeks, they had a level of IgG antibodies against PT more than 125 IU /ml (= 18 VE), and they were vaccinated more than 12 months ago.

Table 3
Children vaccinated with whole-cell vaccine

Period of time after last vaccination	n=74	Positive due to vaccination	Positive due to infection	Negative
1–12 months	24	10	0	14
13–35 months	31	3	4	24
36–42 months	19	1	1	17

In the whole sample of 122 children whose sera were examined 1–42 months after at least 3 doses of vaccination, only 37 children (30 %) had positive levels of antibodies against PT

DISCUSSION

The exact mechanism underlying immunity to pertussis is lacking. Presumably, the immunity to pertussis involves multiple humoral and cellular responses that are not directed against a single protective antigen (*8*). In our study, we focused on the examination of antibodies against PT, the specific marker of pertussis infection and antigen with no described cross-reactivity. The results presented show that the immunogenicity of the acellular and the whole-cell vaccine is equal, and lower than was expected. Similar conclusions were presented in some other studies (*9–12*).

Admittedly, due to the fact that acellular vaccines have been provided free of charge since 01/2007, we still have a low number of children with the complete vaccination against pertussis only with the acellular vaccine.

As it was suggested before, despite the high vaccination coverage in the Czech Republic, an increasing number of cases of pertussis has been reported in recent years. There exist many probable explanations.

First of all, due to the extensive use of antipertussis vaccines since the 1950s, it is conceivable that vaccine-induced immunity has affected the evolution of BP. An antigenic divergence was observed in the surface-associated toxins – pertactin and PT (*13*).

Secondly, it has been demonstrated worldwide that postvaccinal antibodies remain for only a limited period of time (*11, 15*). The results of our study imply that the number of seropositive children declines rapidly in relation to the period of time after the last vaccination. Even after a one-year interval from the last vaccination we observed statistically significant differences in the number of children with positive levels of anti-PT antibodies. In population studies it was summarised that the lowest seropositivity prevalence is in children at preschool and school ages and that a high number of seropositive children is recorded in adolescence, thus suggesting exposure to infection (*4, 11*). Similarly, we observed low levels of antibodies in children of preschool age (36–42 months after the vaccination).

The infection of BP in vaccinated children/adults may not induce the typical clinical symptoms. Frequently it occurs only under the picture of intermittent chronic cough, and is therefore often misdiagnosed. Adolescents and young adults present an important reservoir of pertussis in the population that could be potentially transmitted to young infants and cause a life-threatening disease. Moreover, although the passive transplacental passage of IgG antibodies against pertussis is sufficient, newborns lack satisfactory levels of specific anti-PT antibodies (*16*).

Finally, the increasing incidence of pertussis relates to the migration of population from countries with lower vaccination coverage.

In conclusion, this study was presented to stimulate further investigation in this area. It is essential to examine more sera of children vaccinated with the acellular vaccine after a longer period of time, in order to evaluate the limit of preservation of IgG antibodies against PT.

In relation to the lack of vaccination-induced immunity in the adolescent and adult populations (due to the rapid decline of antibodies after the vaccination), it is absolutely important to involve pertussis in the differential diagnosis of chronic cough and to treat the disease with macrolide antibiotics. The most discussed problem about pertussis vaccination is the introduction of a booster dose with the acellular vaccine at approximately the age of nine (*17, 18, 19*). It would be rewarding to consider this topic in the Czech Republic, similar to some other countries.

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